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# Pleistocene range shifts, refugia and the origin of widespread species in Western Palearctic water beetles

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## ABSTRACT

Quaternary glacial cycles drove major shifts in both the extent and location of the geographical ranges of many organisms. During glacial maxima, large areas of central and northern Europe were inhospitable to temperate species, and these areas are generally assumed to have been recolonized during interglacials by range expansions from Mediterranean refugia. An alternative is that this recolonization was from non-Mediterranean refugia, in central Europe or western Asia, but data on the origin of widespread central and north European species remain fragmentary, especially for insects. We studied three widely distributed lineages of freshwater beetles (the *Platambus maculatus* complex, the *Hydraena gracilis* complex, and the genus *Oreodytes*), all restricted to running waters and including both narrowly distributed southern endemics and widespread European species, some with distributions spanning the Palearctic. Our main goal was to determine the role of the Pleistocene glaciations in shaping the diversification and current distribution of these lineages. We sequenced four mitochondrial and two nuclear genes in populations drawn from across the ranges of these taxa, and used Bayesian probabilities and Maximum Likelihood to reconstruct their phylogenetic relationships, age and geographical origin. Our results suggest that all extant species in these groups are of Pleistocene origin. In the *H. gracilis* complex, the widespread European *H. gracilis* has experienced a rapid, recent range expansion from northern Anatolia, to occupy almost the whole of Europe. However, in the other two

groups widespread central and northern European taxa appear to originate from central Asia, rather than the Mediterranean. These widespread species of eastern origin typically have peripherally isolated forms in the southern Mediterranean peninsulas, which may be remnants of earlier expansion-diversification cycles or result from incipient isolation of populations during the most recent Holocene expansion. The accumulation of narrow endemics of such lineages in the Mediterranean may result from successive cycles of range expansion, with subsequent speciation (and local extinction in glaciated areas) through multiple Pleistocene climatic cycles.

**Keywords:** glacial refugia, Dytiscidae, Hydraenidae, Quaternary glaciations, range expansion, Mediterranean Peninsulas, Central Asia

## 1. Introduction

The Quaternary was a period of drastic cyclical climatic changes, with multiple glacial–interglacial periods, ultimately driven by variations in the earth’s orbit known as Milankovitch cycles. Milankovitch–driven climate oscillations led to large changes in the size and location of the geographic distribution of many species, in some cases resulting in speciation due to the higher probability of isolation of small populations in areas under new selection regimes (Dynesius & Jansson, 2000). These Pleistocene climatic oscillations and the subsequent shifts in ecological conditions, with the repeated fragmentation of populations during glacials and interglacials, have long been hypothesised to have driven the origin of most extant Holarctic species (e.g. Rand, 1948; Mayr, 1970).

Pleistocene climatic changes were especially drastic in northern latitudes of the Palearctic region, since during the Last Glacial Maximum the European ice sheet covered most areas north of 52°N, with permafrost north of 47°N (Dawson, 1992). Large areas of central and northern Europe therefore became inhospitable to temperate taxa during glacials; in stark contrast to the Mediterranean peninsulas, which retained more temperate climate and vegetation (e.g. Huntley, 1988; Bennett et al., 1991). However, despite the fact that most of central and northern Europe and regions of Asia at similar latitudes were exposed to extremely cold conditions (Dawson, 1992), there were areas on the slopes of mountain ranges and along river valleys where moister conditions prevailed (Soffer, 1990), allowing the local survival of temperate biota in these northern/eastern refugia (e.g. Stewart et al., 2009; Schmitt & Varga, 2012).

Two main scenarios could account for the origin of the current central and northern European fauna. Firstly, there is the traditional model of postglacial range expansion from Mediterranean refugia (e.g. Hewitt, 2000), in which central and northern Europe were colonized by northward range expansions mainly from the Iberian, Italian, Balkan and Anatolian peninsulas at the end of the last glaciation. According to this model, populations of most European species were confined to refugial areas in southern Mediterranean peninsulas during glacial maxima, from which they would have re-colonized the continent during interglacials (although Hewitt (2000) also recognised the important role of the Carpathians as providing potential refugia). Whilst such a scenario is well established for some taxa, it is not ubiquitous. A second possibility is that the colonization of central and northern Europe at the end of the Last Glacial was from non-Mediterranean source areas in eastern Europe and Asia (Bilton et

al., 1998). According to this view, the isolation of the Mediterranean peninsulas during glacial cycles led to speciation, preventing gene flow with the new colonisers of central and northern Europe during subsequent interglacials. For taxa conforming to this model, southern peninsulas are centres of endemism rather than being a source of colonists (Bilton et al., 1998; Schmitt & Varga, 2012).

Such biogeographical isolation of Mediterranean peninsular populations has been suggested previously for small mammals (Bilton et al., 1998) and some insects (e.g. Cooper et al., 1995). Amongst aquatic Coleoptera, the absence of fossil remains of southern species in the abundant central and northern European Quaternary subfossil record (Abellan et al., 2011) supports a view of Mediterranean peninsulas as areas of endemism, rather than significant sources of postglacial colonists. Data from extant species also suggest that current southern endemics have not contributed to the diversity of northern areas (e.g. *Hydrochus* (Hydrochidae), Hidalgo-Galiana & Ribera, 2011; or *Enicocerus* (Hydraenidae), Ribera et al., 2010). Some central and northern European species may have had their origin in Mediterranean peninsulas, but in such cases it appears that the taxa concerned were those whose refugia were located in the northernmost areas of the peninsulas, on the margins of deglaciated areas (e.g. Ribera et al., 2010 for *Enicocerus*, and García-Vázquez & Ribera, 2016 for *Deronectes*), successful expansion possibly being aided by physiological adaptations in such species (Calosi et al., 2010; Cioffi et al., 2016).

Despite increased understanding of the evolution of the European insect fauna in recent decades, data on the origin of widespread central and northern European species, which should have necessarily experienced recent expansions of their geographical ranges, remain severely limited. Here we study a suite of such species, using molecular phylogeographic data to clarify their temporal and geographic origin and to better understand the role of the Pleistocene glacial cycles in driving their diversification. We examined species groups from three genera of freshwater beetles, in two different families, whose representatives colonised water independently: 1) the *Hydraena gracilis* complex (“*Haenydra*” lineage, family Hydraenidae); 2) the *Platambus maculatus* complex (family Dytiscidae) and (3) *Oreodytes sanmarkii* (C.R. Sahlberg) and *O. davisii* (Curtis) (family Dytiscidae). All taxa concerned are typical of running waters, and include both widespread European and narrowly distributed southern endemic species (Trizzino *et al.*, 2013; Nilsson & Hájek, 2017a,b). They do, however, differ in

functional traits and evolutionary histories (see below), facts which contribute to the generality of our conclusions.

Using a combination of mitochondrial and nuclear data we reconstruct the phylogenetic relationships, age and geographical origin of the western Palaearctic species of these three widely distributed lineages, to better understand the effects of Quaternary glacial cycles on their diversification and current distributions.

## 2. Material and Methods

### 2.1. Taxonomic background and taxon sampling

#### a) *Hydraena gracilis* complex

The genus *Hydraena*, currently with ca. 900 species distributed worldwide (Trizzino et al., 2013) is the largest genus within the family Hydraenidae and probably the most diverse amongst the aquatic Coleoptera (Jäch & Balke, 2008). Within *Hydraena*, the “*Haenydra*” lineage includes ca. 90 species with a north Mediterranean distribution (Trizzino et al., 2013). They are usually found in clean, fast flowing waters, often in mountain streams, from the Iberian Peninsula to Iran and the Urals, but are absent from North Africa (Ribera et al., 2011; Trizzino et al., 2011; Trizzino et al., 2013; Jäch, 2015). Many species of this lineage have very restricted distributions, often limited to a single valley or mountain system, but there are also a few species with very wide geographical ranges.

In this work we focus on the most widespread species of “*Haenydra*”, *Hydraena gracilis* Germar and its closest relatives in the *H. gracilis* complex *sensu* Jäch (1995), which includes seven recognised species and one subspecies (Trizzino et al., 2013). *Hydraena gracilis* is widely distributed across almost the whole of Europe, ranging from southern France eastwards to Ukraine and northwards to Finland, including the British Isles (Fig. 1). Previous molecular studies, albeit on a limited number of specimens (Ribera et al., 2011), suggested that despite its widespread distribution, genetic differences across its geographic range were minimal. Jäch (1995), however, found morphological differences between specimens from the Balkans and the rest of Europe, supporting the recognition of the subspecies *H. gracilis balcanica* d’Orchymont. *Hydraena gracilis* is absent from the Iberian and Anatolian peninsulas, where it is replaced by different species of the complex (Fig. 1). *Hydraena gracilidelphis* Trizzino, Valladares, Garrido & Audisio is the westernmost species of this group, endemic to the Iberian Peninsula (mainly in the north but with some records

in the southwest) and the French Pyrenees (Trizzino et al., 2012). The Anatolian Peninsula and adjacent areas are occupied by three species: *H. anatolica* Janssens distributed in northern and eastern Anatolia and parts of the Caucasus and northwestern Iran; *H. graciloides* Jäch in northern Turkey; and *H. crepidoptera* Jäch known only from two northern Turkish provinces (Kastamonu and Sinop). The other two species of the complex, *H. nike* Jäch and *H. elisabethae* Jäch, are endemic to two Aegean islands; Samothraki and Thassos respectively (Trizzino et al., 2013).

We studied a total of 48 specimens from five of the seven species of the *H. gracilis* complex (we could not obtain fresh specimens of the two Aegean Island endemics) from 37 different localities, covering the full geographical range of the studied species (Fig. 1; Table S1). As outgroups we used three closely related species of the wider *H. gracilis* lineage within “*Haenydra*” (Trizzino et al., 2011; Table S1).

#### b) *Platambus maculatus* complex

The genus *Platambus* contains 66 recognised species (Nilsson & Hájek, 2017a) and has a wide distribution, being present in the Palearctic, Nearctic, Neotropical and Oriental regions, and is currently divided into eight species-groups (Nilsson & Hájek, 2017a). Amongst these the *P. maculatus* group - as defined by Nilsson (2001) - is the largest, with 24 species distributed across Asia and Europe. In a molecular phylogeny of Agabinae Ribera et al. (2004) recovered a paraphyletic *Platambus*, with the *P. maculatus* group separated from other Asian and American species.

Here we focus on the most widespread species of the group; *P. maculatus* (Linnaeus) and its closest relative, *P. lunulatus* (Fischer von Waldheim), which we refer to as the *Platambus maculatus* complex. *Platambus maculatus* has a wide Palearctic distribution, from western Iberia to northern Iran and Mongolia (including Italy, the Balkans and Anatolia), Scandinavia and the British Isles (Nilsson & Hájek, 2017b; Fig. 2). The species has a very variable elytral pattern, which led to the description of many forms all of which are currently considered synonyms of *P. maculatus* (Nilsson & Hájek, 2017a,b). Most conspicuous amongst these is *P. maculatus* “*graellsii*” (Gemminger & Harold) from the northwest and centre of the Iberian Peninsula (Millán et al., 2014). The other species of the complex, *P. lunulatus*, is distributed from the Anatolian Peninsula and parts of the Caucasus and Middle East to Egypt (Karaman et al., 2008; Nilsson & Hájek, 2017b) (Fig. 2).

We sequenced 106 specimens from 67 different localities of the two recognised species of the *P. maculatus* complex, including the "*graellsii*" form (Fig. 2; Table S1). As outgroups we used seven specimens from different species of the *Platambus maculatus* group, as defined in Nilsson & Hájek (2017b).

c) European species of the genus *Oreodytes*

The Holarctic genus *Oreodytes* Seidlitz contains 30 recognised species, four of them split into two subspecies (Fery, 2015; Nilsson & Hájek, 2017a). *Oreodytes* species live in cold streams or lakes margins, generally at high altitude or latitude (Balfour-Browne, 1940; Zack, 1992; Nilsson & Holmen, 1995). The genus is distributed in the Palearctic and Nearctic regions, with six species occurring in Europe. In a previous study, Ribera (2003) recovered a paraphyletic *Oreodytes*, although with low bootstrap support. *Oreodytes* was divided into two lineages corresponding to the main distinction in body size and shape, i.e. larger and more elongate species (including *O. davisii*) versus smaller and rounder ones (including *O. sanmarkii*).

Of the six European species of *Oreodytes*, the most widespread is *O. sanmarkii*, distributed over large parts of the Palearctic from the Iberian Peninsula to the Russian Far East, and reaching the Nearctic in northern Canada (Larson et al., 2000; Nilsson & Hájek, 2017a,b) (Fig. 3). In southern Europe, the species is known from the Iberian Peninsula, northern provinces of Italy and the Balkans south to Bulgaria and Macedonia (Fery, 2015; Nilsson & Hájek, 2017b). The species shows a high level of variability in colouration over its large distributional range (e.g. Larson, 1990) but only one subspecies, *O. sanmarkii alienus* (Sharp), endemic to the Iberian Peninsula, is currently recognised (Balke, 1989; Nilsson & Hájek, 2017a,b). Also with a wide Palearctic distribution is *O. davisii*, known from the British Isles to Ukraine and the Caucasus, including Scandinavia, the Mediterranean peninsulas and Turkey (Nilsson & Hájek, 2017b) (Fig. 3). In the case of this species an Iberian form has also been recognised as a subspecies on morphological grounds, *O. davisii rhianae* Carr (Carr, 2001; Nilsson & Hájek, 2017a,b). The other European species of *Oreodytes* are *O. septentrionalis* (Gyllenhal), distributed from the Iberian Peninsula to eastern Siberia and Mongolia (Nilsson & Hájek, 2017b); *O. alpinus* (Paykull), with a northern Palearctic distribution, being present from lochs in northern Scotland (Foster, 1992) and Scandinavia to Kamchatka in the Russian far East (Nilsson & Kholin, 1994); *O. meridionalis* Binaghi



& Sanfilippo, endemic to the southern Apennines (Rocchi 2007; Nilsson & Hájek, 2017b); and the recently described *O. angelinii* Fery from Greece (Fery, 2015).

We studied 58 specimens from 35 different localities of all the European species of *Oreodytes* with the exception of the Italian endemic *O. meridionalis* and the newly described *O. angelinii*, with a focus on *O. davisii* and *O. sanmarkii* and the two Iberian subspecies (*O. d. rhianae* and *O. s. alienus* respectively). We also included in the analysis 16 specimens of different Asian and American species of the genus as outgroups (Fig. 3; Table S1).

## 2.2. DNA extraction and sequencing

Specimens were collected and preserved in absolute ethanol directly in the field. We extracted the DNA non-destructively with commercial kits (mostly "DNeasy Tissue Kit", Qiagen GmbH, Hilden, Germany and "Charge Switch gDNA Tissue Mini Kit", Invitrogen, Carlsbad, CA, USA) following the manufacturers' instructions. Specimens and DNA extractions are deposited in the collections of the Institut de Biologia Evolutiva, Barcelona (IBE), Museo Nacional de Ciencias Naturales, Madrid (MNCN) and Natural History Museum, London (NHM). We obtained seven gene fragments from six different genes (four mitochondrial and two nuclear) in five different amplification reactions (see Table S2 for primers and typical sequencing reactions): (1) 5' end of the Cytochrome Oxidase Subunit 1 gene (the barcode fragment; Hebert et al., 2003, COI-5'); (2) 3' end of Cytochrome Oxidase Subunit 1 (COI-3'); (3) 5' end of 16S rRNA plus tRNA transfer of Leucine plus 3' end of NADH subunit 1 (*nad1*) (16S and *nad1* respectively); and internal fragments of the nuclear genes (4) Histone 3 (H3) and (5) Wingless (Wg). Due to their lower variability, the nuclear markers were only sequenced from representative specimens according to geographical and topological criteria. For each amplification reaction we obtained both forward and reverse sequences. In some specimens, due to difficulties in amplification, we used internal primers for the COI-3' sequence, obtaining two fragments of 400 bp each (Table S2). PCR products were purified by standard ethanol precipitation and sent to external facilities for sequencing. DNA sequences were assembled and edited using the Geneious 6 software (Biomatters Ltd, Auckland, New Zealand). Ambiguous calls in the nuclear genes were coded as "N"s. New sequences (561) have been deposited in GenBank with accession numbers LT855666-LT856230 (Table S2).

### 2.3. Phylogenetic and divergence time analyses

Edited sequences were aligned with MAFFT v.6 using the G-INS algorithm and default values for other parameters (Katoh & Toh, 2008). We included sequences obtained from the literature (GenBank and BOLD databases) in some analyses to increase geographical coverage and possible genetic variation not covered by our sampling.

In the phylogenetic analyses we employed six partitions, corresponding to the gene fragments COI-5', COI-3', 16S, nad1, H3 and Wg, and used Partition Finder 1.1.1 (Lanfear et al., 2012) to estimate the best-fitting models of nucleotide substitution for each partition separately, using AIC (Akaike Information Criterion). We considered the two fragments of the COI gene separately due to the uneven taxonomic coverage (Table S1). To infer the phylogeny of the three groups, and estimate divergence dates amongst species, we used Bayesian methods implemented in Beast 1.8 (Drummond et al., 2012). For the analyses we included both mitochondrial and nuclear markers and implemented the closest available evolutionary model to those selected by Partition Finder. We used a Yule speciation model and ran two analyses to determine which clock model (strict or lognormal relaxed) best fitted the data. As there are no fossils or unambiguous biogeographic events that could be used to calibrate the phylogeny of the studied groups, we applied published estimations of a-priori rates for the same genes in related groups of beetles. For the *Platambus maculatus* complex and the genus *Oreodytes* (both Dytiscidae) we used a rate of 0.013 substitutions/site/MY (SD 0.002) for protein coding genes and 0.0016 substitutions/site/MY (SD 0.0002) for 16S, obtained for the related family Carabidae for the same combination of mitochondrial protein coding and ribosomal genes (Andújar et al., 2012). For the *Hydraena gracilis* complex we used a rate of 0.015 and 0.006 substitutions/site/MY (SDs 0.002 and 0.0002) for the COI-3'+nad1 and 16S partitions respectively, obtained for the related Leiodidae (Cieslak et al., 2014). Clock rates of H3 and Wg were left with uniform priors due to the absence of any suitable estimations of the evolutionary rate for these nuclear genes. We executed two independent analyses with the same settings, running 100 million generations (saving trees every 5,000) or until analyses converged and the number of trees was sufficient according to Effective Sample Size (ESS) values, as measured with Tracer v1.6 (Rambaut et al., 2014). The maximum clade credibility tree of the two runs was compiled with Tree Annotator v1.8 (Drummond et al., 2012) and visualized with FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). For selection of the best molecular

clock we used the modified Akaike Information Criterion (AICM) with the moments estimator (Baele et al., 2012), as implemented in Tracer v1.6, with 1,000 bootstrap replicates.

To test for potential topological discordances between mitochondrial and nuclear data we analysed the nuclear genes only, applying the best clock and the same settings as in the combined (mitochondrial and nuclear) analysis.

We also analysed the combined matrix (mitochondrial and nuclear) using maximum likelihood (ML) methods. ML analyses were performed in RAxML v.7.4.2 (Stamatakis et al., 2008) as implemented in RAxML GUI v 1.3.1 (Silvestro & Michalak, 2012). We selected the best tree out of 100 searches using the GTR+G as an evolutionary model with the same six partitions as in the Bayesian analysis. Node support was estimated with 1,000 bootstrap replicates.

#### 2.4. Demographic analyses

To study population history for each of the three groups separately we estimated the population coalescent model that best fitted the data. We used the mitochondrial sequences only and no outgroups. In the analysis for the *H. gracilis* complex we only included specimens of *H. gracilis* and *H. anatolica*, whilst only specimens of *P. maculatus* (including the "*graellsii*" form) and *O. sanmarkii* were included in the analyses for *Platambus* and *Oreodytes* respectively. The datasets were divided into four partitions, corresponding to each mitochondrial gene (COI-5', COI-3', 16S and nad1) and the same settings used as in the topological analyses, including evolutionary models selected by Partition Finder and the best molecular clock for each group. To identify the best demographic model we ran four analyses including Constant Size, Exponential Growth, Logistic Growth and Expansion Growth coalescence models. For model selection of the best coalescent analyses we used the modified Akaike Information Criterion (AICM) with the moments estimator as implemented in Tracer v1.6, with 1,000 bootstrap replicates. We also computed Bayesian skyline plots (Drummond et al., 2005) for each group, to reconstruct variation in effective population sizes through time.

### 3. Results

There were no length differences in protein coding genes, and the length of ribosomal genes in the ingroup ranged between 784-786 bp in the *H. gracilis* complex and 796-797 bp in the *P. maculatus* complex and the genus *Oreodytes*. The best

evolutionary models, as selected by Partition Finder for each individual partition (Table S3), were implemented in the Bayesian analysis for the *H. gracilis* complex and the species of the genus *Oreodytes*. In the *P. maculatus* complex, when the more complex models were applied the analyses did not converge adequately for both the relaxed lognormal and strict clocks, and in consequence we applied an HKY+I+G model to each partition.

### 3.1. *Hydraena gracilis* complex

The nucleotide alignment matrix showed no variability in the two nuclear genes, which were therefore excluded from subsequent analyses. The analysis using a lognormal relaxed clock did not converge adequately so we applied a strict molecular clock model.

The ultrametric tree obtained with the combined mitochondrial and nuclear data estimated a recent origin and diversification of the *H. gracilis* complex at ca. 0.3 Ma (95% confidence interval (c.i.) 0.4-0.2 Ma) (Fig. 4), with strongly supported monophyly. The complex was divided into two major clades, one included the Iberian *H. gracilidelphis* plus all specimens from the easternmost populations identified as *H. anatolica* (eastern Anatolia, the Caucasus and Iran), and the other was formed by two groups, one containing specimens of *H. anatolica* and *H. crepidoptera* from northern Turkey and the other with the widespread European *H. gracilis*, including the subspecies *H. gracilis balcanica* and two Turkish specimens of *H. anatolica* and *H. graciloides*. In the latter clade, specimens from the most southeasterly populations occupied basal positions, with the more western and northern populations nested within them (Fig. 4). The lineage with the eastern specimens of *H. anatolica* had a poorly supported position between the two remaining main clades of the complex, which differed between analyses. Bayesian analysis (Fig. 4) recovered the eastern lineage as sister to the Iberian *H. gracilidelphis*, whilst ML analysis (Fig. S1) linked the eastern lineage to the other clade, as sister to *H. gracilis* and the Turkish westernmost populations, but always with low support.

In the coalescence analyses a constant size model was preferred over exponential growth (Table 1) (logistic and expansion models did not converge adequately). The Bayesian Skyline Plot showed a continuous, slight increase in population size, with a marked increase at ca. 50,000 years BP and a slight decrease towards the present (Fig. 5A).

### 3.2. *Platambus maculatus* complex

The relaxed lognormal was significantly better than the strict molecular clock, and was therefore implemented in the Bayesian analyses (Table 1). The temporal origin of the *P. maculatus* complex was estimated to be in the Middle Miocene (ca. 12 Ma) (Fig. 6), although the split between the two extant species (*P. maculatus* and *P. lunulatus*) occurred in the Messinian (ca. 6.5 Ma, c.i. 9.3-4.1 Ma). Extant intraspecific variability dates from the Pliocene-Pleistocene boundary (2.5-3.5 Ma). The ultrametric tree obtained from Bayesian analysis using the combined mitochondrial and nuclear matrix strongly supported the monophyly of the two recognised species (Fig. 6).

*Platambus maculatus* was divided into three clades, one with specimens with a predominantly western distribution (northern Spain, France and the British Isles, including specimens from some Scottish lochs, these forming a monophyletic lineage); a second clade with specimens from easterly populations (central Europe, Scandinavia, the Balkans, Anatolia and the Middle East); and a third clade including specimens of the Iberian "*graellsii*" form plus *P. maculatus* from northern Italy and the two sampled specimens from one of the localities in the Pyrenees (PIR11, Fig. 6; Table S1). The relationships between these three lineages were not well supported, and varied between analyses. In the Bayesian analysis using only nuclear markers (Fig. S2), all specimens of *P. maculatus* "*graellsii*" plus the northern Italian and PIR11 *P. maculatus* were included within the western clade. In the ML analysis, on the contrary (Fig. S3), we recovered the northern Italian specimens of *P. maculatus* as sister to all other specimens, which were split into three poorly supported groups (western clade, eastern clade and the "*graellsii*" form plus the PIR11 *P. maculatus* referred to above).

None of the four coalescence models converged adequately, but the Bayesian Skyline Plot showed a nearly constant effective population size until ca. 15,000 years BP, with a recent increase (Fig. 5B).

### 3.3. *European Oreodytes* species

A relaxed lognormal clock was preferred over a strict molecular clock, and was implemented in the Bayesian analyses (Table 1). Preliminary results showed the existence of two group of species, one closely related to *O. sanmarkii* (the *O. sanmarkii* group) and the other to *O. davisii* (the *O. davisii* group), the later including the European *O. septentrionalis* and *O. alpinus*. These two clades were constrained as

monophyletic in subsequent Bayesian analyses without outgroups. *Oreodytes alpinus* was nested within *O. davisii* in the analysis using the combined mitochondrial and nuclear matrix (Fig. 7), forming a clade with the eastern Palaearctic *O. mongolicus* (Brinck). In the analysis using only nuclear data, *O. alpinus* and *O. mongolicus* were also sisters, but both sister to *O. davisii* (Fig. S4)

Divergence time analysis (Fig. 7) dated the separation between the *O. davisii* and *O. sanmarkii* groups to the Oligocene (ca. 27 Ma, c.i. 36-20 Ma) and diversification within them to the Lower Miocene (ca. 22 Ma, c.i. 30-15 Ma) for the *O. sanmarkii* group and the Middle Miocene for the *O. davisii* group (ca. 13 Ma, c.i. 18-10 Ma) (Fig. 7). The sister species of the clades *O. davisii*+*O. alpinus*+*O. mongolicus* and *O. sanmarkii* were the North American *O. snoqualmie* (Hatch) and *O. obesus* (LeConte), respectively. Intraspecific variation in both *O. davisii* and *O. sanmarkii* was of Pleistocene origin (1.5-2.0 Ma) (Fig. 7).

We found no clear phylogeographical signal in *O. davisii*, irrespective of the method of analysis (Bayesian or ML). In contrast, *O. sanmarkii* was divided into two clades with a clear geographical pattern, one including specimens with an eastern distribution, from Mongolia to central Europe, and the other including mainly western specimens, from the Iberian Peninsula, Italy and the British Isles, although also including some individuals from the Carpathians. Within the western clade, Iberian populations from the Pyrenees were separated from those from Portugal and central and northwestern Spain, the latter identified as the subspecies *O. sanmarkii alienus*. This difference was more pronounced in the ML analysis (Fig. S5; although with poor support) and in the analysis using only nuclear data (Fig. S4), which recovered *O. s. alienus* as sister to remaining *O. sanmarkii*.

In the case of other European species of the group, we found a deep divergence between Mongolian and European specimens of *O. septentrionalis*, estimated to have occurred during the late Miocene (ca. 5.6 Ma, c.i. 8.3-3.5 Ma; Fig. 7).

The expansion growth coalescence model performed better than logistic or exponential ones (Table 1); the constant size model failing to converge adequately. The Bayesian Skyline Plot showed that effective population size remained constant until relatively recently, with a sharp increase ca. 10,000 years BP (Fig. 5C).

#### 4. Discussion

Our results emphasise the fact that patterns of evolutionary diversification, biogeographical history and range expansion can differ significantly, even when comparing taxa occupying the same broad habitat type in the same region. The three groups of water beetles examined here have all diversified during the Plio-Pleistocene, and been subject to the same historical climatic shifts, but despite this their colonization history and demography differ considerably.

#### 4.1. *Hydraena gracilis* complex

According to our results the *H. gracilis* complex has a recent, Pleistocene origin, with the widespread *H. gracilis* showing extreme genetic homogeneity throughout its range. This suggests a very recent expansion, in agreement with preliminary results for this and other species of the "*Haenydra*" lineage (Ribera et al., 2011). The existence of narrow endemics around the periphery of the range of the complex, both in the far west (Iberian Peninsula) and east (Anatolia, Azerbaijan and Iran) is consistent with successive cycles of range expansion within the complex, followed by local extinctions, most likely during the glacial periods. During such times, remaining populations would have been isolated in refuges, resulting in the divergence of *H. gracilidelphis* in the Iberian Peninsula and *H. anatolica*, *H. graciloides* and *H. crepidoptera* in Turkey and the Middle East - a scenario consistent with the model for diversification of this group proposed by Ribera et al. (2011).

On current data it was not possible fully to resolve the relationships of eastern Anatolian and Iranian populations of *H. anatolica*, which may be the remnants of earlier diversification cycles (as is the case for *H. gracilidelphis* in the west) or represent early isolates of the most recent range expansion. The separation between western and eastern populations in *H. anatolica* suggests that the Anatolian Diagonal, a mountain range running from north-eastern to south-western Anatolia, acts as an effective barrier to gene flow between western and eastern regions, a pattern observed in many Turkish taxa (Gündüz et al., 2007). The basal position of northern and central Turkish populations of the *H. gracilis* group suggests a second range expansion from these areas, crossing the straits to the Balkans and expanding to western and northern Europe, resulting in the current widespread European *H. gracilis*. This pattern of expansion has been observed in other taxa, including insects (e.g. grasshoppers, Korkmaz et al., 2014) suggesting that Anatolia was an important glacial refugium, which contributed to the recolonization of Europe in some lineages (Ansell et al., 2011; Hewitt, 1996; Rokas et

al., 2003). Such expansion from Anatolia to the Balkans may have been possible through a substantial decrease in sea level during glacial periods (Aksu et al., 1999; Ergin et al., 2007), which resulted in a large part of the Sea of Marmara becoming dry land through which terrestrial and freshwater taxa may have dispersed.

#### 4.2. *Platambus maculatus* complex

Although the precise geographic origin of the *P. maculatus* complex remains uncertain, their closest relatives are distributed in eastern and central Asia (Nilsson, 2015; Nilsson & Hájek, 2017b). Subsequent to the western range expansion from central Asia, one lineage differentiated in Asia Minor and Anatolia, resulting in *P. lunulatus*, and the other Europe, resulting in *P. maculatus*.

Range expansion in *P. maculatus* followed a clear geographical pattern, with western and eastern populations falling into two well-supported clades (Fig. 6). An exception here is the uncertain position of specimens from northern Italy and the Iberian "*graellsii*" form, both of which show appreciable morphological differences in elytral pattern and sculpture. Northern Italian specimens have long been recognised as amongst the largest, most convex and shiny of this species (Sharp, 1882; Balfour-Browne, 1940) (Figs 6, 8), whilst populations from central Iberia were originally described as a distinct species (*Agabus glacialis* Graells, subsequently changed to *A. graellsii*), but then reduced to a variety and finally synonymised with *P. maculatus* (Nilsson & Hájek, 2017a,b). They have a more reddish coloration than the typical forms of *P. maculatus*, with a poorly defined colour pattern and a very dense and deep microsculpture, giving their dorsal surface a dull, rough appearance (Sharp, 1882) (Figs 6, 8). Both central Iberian and north Italian populations are at the periphery of the main range of the species, in a situation similar to that for *H. gracilidelphis* and the Iberian *Oreodytes* (see below). In *Platambus* our data are, however, inconclusive regarding the origin of these forms, which could have been isolated in the Iberian and Italian Peninsulas as remnants of an earlier range expansion in the complex, or result from incipient isolation during the most recent expansion event.

Within the western clade, specimens from some oligotrophic Scottish lochs, also at the periphery of the species main range, formed a monophyletic lineage. This is most remarkable, as they were, together with *P. maculatus* "*graellsii*" and the northern Italian populations, the only forms highlighted in the monumental revision of Sharp (1882). Scottish loch animals were described as being the smallest of the species, flatter in



shape, with a duller surface and reduced yellow markings on the elytra (Sharp, 1882; Balfour-Browne, 1940) (Figs 6, 8). However, three specimens from Loch Eck, with a similar morphology (although with less marked differences) were not placed in this clade, but amongst other lineages within the wider western clade (Fig. 6; Table S1). We performed additional analyses, including sequences obtained from public databases, to identify specimens with similar haplotypes to those of Scottish lochs, and found a single northern Swedish sequence which nested within the Scottish clade (obtained from Bergsten et al., 2012). The external morphology of this specimen was within the typical range of *P. maculatus* however, and other beetles from the same locality had similar morphology, and COI haplotypes which nested within the western continental lineage. The nuclear markers used did not have enough resolution to determine if such incongruences are the result of introgression, but in any case our results suggest that some Scottish populations, and perhaps others in northern Europe, could be remnants of an early northward range expansion in *P. maculatus*.

#### 4.3. European species of the genus *Oreodytes*

Most species of the genus *Oreodytes*, including the sister species of both *O. davisii* and *O. sanmarkii* (*O. snoqualmie* and *O. obesus* respectively) are distributed in the western United States and Canada (Larson, 1990; Larson et al., 2000). The geographical origin of European taxa therefore seems to have been via range expansion through Beringia and Asia. In the case of *O. davisii* this expansion has not left any apparent phylogeographical structure in the studied markers. In the combined analyses (largely driven by mitochondrial data) *O. alpinus* and *O. mongolicus* were nested within *O. davisii*, despite considerable morphological differences between these species (Shaverdo & Fery, 2006; Foster & Friday, 2011). In contrast, the analysis using only nuclear data clearly separated both species from *O. davisii*, consistent with past mitochondrial introgression between populations of these closely related species who are likely to have shared the same broad Pleistocene refugia. Such a situation has been reported from a number of other taxa (e.g. Berthier et al., 2006; Nichols et al., 2012) including aquatic beetles (Hidalgo-Galiana et al., 2014, García-Vázquez et al., 2016).

In *O. sanmarkii* there is a more clearly defined phylogeographic structure, with extant western and eastern clades dating from the middle Pleistocene. This suggests an early origin of European populations, with subsequent isolation of these in different eastern and western refugia. Both *O. davisii* and *O. sanmarkii* are very cold resistant (in

the Pyrenees both can be active in winter in partly frozen streams, I. Ribera unpublished observations), likely able to survive in relatively high northern latitudes during glacial cycles, something which may have favoured local persistence in cryptic northern refugia (see below).

Similarly to the *P. maculatus* and *H. gracilis* complexes, the only recognised forms within *O. davisii* and *O. sanmarkii* are the Iberian *O. davisii rhianae* and *O. sanmarkii alienus* respectively, both occurring west and south of the Ebro valley (Balke, 1989; Carr, 2001). In the analysis of nuclear data, and also ML analysis of the combined dataset, the sequenced specimens of *O. sanmarkii alienus* formed a monophyletic group, although in a relatively unsupported position with respect to other *O. sanmarkii*. Irrespective of its taxonomic status it seems that *O. sanmarkii alienus* is relatively isolated genetically from other European populations, reinforcing the pattern of peripheral isolation across the ranges of widespread European taxa. Although difficult to assess without molecular data, the two missing European species of *Oreodytes* in our study, *O. meridionalis* and *O. angelinii*, are also most likely recent peripheral isolates in Mediterranean peninsulas. Thus, *O. meridionalis*, from the central and southern Apennines (Rocchi, 2007) was considered to be a synonymy of *O. davisii* by Franciscolo (1979) due to their close external morphology. Similarly, *O. angeliini*, from northern Greece, has only been recently recognised as a distinct species by Fery (2015), having been considered within the morphological variability of *O. sanmarkii* by previous authors.

#### 4.4. Concluding remarks: routes of recolonization

Our results show that for some water beetles, as in many other groups, central and northern Europe were recolonized by range expansions from peripheral refugia at the end of the last glaciation. Northern areas of Iberia and Anatolia appear particularly relevant for the taxa studied here, both as sources of recolonists and as cradles for recent, narrow-range endemics. In addition to this classic pattern, however, we also show that some widespread central and northern European species originated not around the Mediterranean basin, but in central Asia, although such taxa still have peripherally isolated forms in the southern Mediterranean peninsulas that in some cases possess divergent haplotypes from those in central and northern Europe. Such species may have colonized northern areas of the continent from cryptic refugia in central/eastern Europe or western Asia during the Holocene. Of particular interest is the possibility of

peripheral refugia not only in the Mediterranean region but also in some areas in the north, as suggested by the Scottish form of *P. maculatus*.

Glacial refugia during cold episodes in Europe were not restricted to the three southern peninsulas, as shown by multiple examples from the Carpathians (Willis et al., 2000; Deffontaine et al., 2005; Kotlík et al., 2006; Sommer & Nadachowski, 2006), and other areas in north and central Europe (Kullman, 1998; Bilton et al., 1998; Stewart & Lister, 2001). Quaternary deposits suggest that a woodland zone existed in the southern foothills of the Carpathian mountains and in sheltered valleys at mid elevations, even during the LGM (Lozek, 2006; Willis et al., 2000). More easterly areas could have also been involved in the colonization of Europe from Asia, including the Caucasus (Massilani et al., 2016). Other potential refugia may have been situated in the Urals, the northern slopes of the Altai, or the Crimean Peninsula (Grichuk, 1984; Hewitt, 1999; Soffer 1990) as well parts of the Ukraine and European Russia (Tarnowska et al., 2016).

In the case of the species studied here, as with other European lotic water beetles (García-Vázquez & Ribera, 2016), interglacial range expansions did not result in the mixing and homogenisation of gene pools, but instead drove the isolation and differentiation of populations at range edges. All the running water beetle lineages studied here are relatively weak dispersers compared to most standing water relatives. All *P. maculatus* examined by Jackson (1952, 1956) had reduced flight muscles and most studied specimens of *O. sanmarkii* have reduced flight muscles, although there is at least one record of a specimen with these fully developed (Foster et al., 2016). *O. davisii* and *H. gracilis* are known to fly (Jäch, 1997; Foster et al., 2016) although have been recorded doing so relatively rarely. It is interesting, however, that these two species are the most genetically homogeneous of the species studied here. This relatively poor dispersal ability begs the question as to how some species are able to expand their ranges to continental scales? The relative genetic homogeneity of widespread species, with genetic differentiation only in peripheral isolates, together with our coalescence data, suggest rapid range expansions over short temporal windows which may have provided optimal ecological conditions for movement between habitat patches. Recently deglaciated areas are likely to have supported a high density of lotic environments, something which may have facilitated the expansion of these beetles. In the Massif Central, Ponel et al. (2016) found an increase in fossil remains of lotic water beetle species immediately after the Last Glacial, but also following the Younger Dryas. This abundance of lotic species was associated with an increase in stream flow resulting

from snow melt during the rapid warming following these two cold periods. When soil formation and sedimentation transformed the landscape, the abundance of lotic species decreased in parallel with an increase in lentic taxa (Ponel et al., 2016). If the same habitat succession happened at larger geographical scales across the continent, it would have facilitated rapid range expansions in lotic species living close to the margins of deglaciated areas, but only for a short time period. As conditions changed, many populations are likely to have become locally extinct, precipitating the genetic isolation of the remainder and in some their eventual speciation (Ribera et al., 2011). The accumulation of narrowly endemic species in lotic lineages may result from successive cycles of range expansion with subsequent speciation and local extinction in glaciated areas over multiple Pleistocene glacial cycles.

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## References

- Abellán, P., Benetti, C.J., Angus, R.B., Ribera, I., 2011. A review of Quaternary range shifts in European aquatic Coleoptera. *Glob. Ecol. Biogeogr.* 20, 87–100.
- Aksu, A.E., Hiscott, R.N., Yaşar, D., 1999. Oscillating Quaternary water levels of the Marmara Sea and vigorous outflow into the Aegean Sea from the Marmara Sea–Black Sea drainage corridor. *Mar. Geol.* 153, 275–302.
- Andújar, C., Serrano, J., Gómez-Zurita, J., 2012. Winding up the molecular clock in the genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions on rate and node age estimation. *BMC Evol. Biol.* 12, 1.
- Ansell, S. W., Stenøien, H. K., Grundmann, M., Russell, S. J., Koch, M. A., Schneider, H., Vogel, J. C., 2011. The importance of Anatolian mountains as the cradle of

623 global diversity in *Arabis alpina*, a key arctic–alpine species. *Ann. Bot.* 108, 241–  
624 252.

625 Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A. V.,  
626 2012. Improving the accuracy of demographic and molecular clock model  
627 comparison while accommodating phylogenetic uncertainty. *Mol. Biol. Evol.* 29,  
628 2157–2167.

629 Balfour-Browne, F., 1940. British water beetles. Vol. 2. Ray Society, London.

630 Balke, M., 1989. Zur identität von *Hydroporus alienus* Sharp, 1872 (Coleoptera,  
631 Dytiscidae). *Elytron* 3, 113–114.

632 Bennett, K.D., Tzedakis, P.C., Willis, K.J., 1991. Quaternary refugia of north European  
633 trees. *J. Biogeogr.* 18, 103–115.

634 Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M.,  
635 Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N., Ribera, I., Nilsson, A.N.,  
636 Barraclough, T.G., Vogler, A.P. 2012. The effect of geographical scale of  
637 sampling on DNA barcoding. *Syst. Biol.* 61, 851–869.

638 Berthier, P., Excoffier, L., Ruedi, M., 2006. Recurrent replacement of mtDNA and  
639 cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis*  
640 *blythii*. *Proc. R. Soc. London B Biol. Sci.* 273, 3101–3123.

641 Bilton, D.T., Mirol, P.M., Mascheretti, S., Fredga, K., Zima, J., Searle, J.B., 1998.  
642 Mediterranean Europe as an area of endemism for small mammals rather than a  
643 source for northwards postglacial colonization. *Proc. R. Soc. London B Biol. Sci.*  
644 265, 1219–1226.

645 Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C., Atfield, A., 2010. What determines a  
646 species' geographical range? Thermal biology and latitudinal range size  
647 relationships in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.*  
648 79, 194–204.

649 Carr, R., 2001. *Oreodytes davisii rhianae* subsp. nov. (Coleoptera: Dytiscidae): an  
650 Iberian subspecies distinct from *O. davisii davisii* (Curtis, 1831). *Entomol. Gaz.*  
651 52, 183–188.

652 Cieslak, A., Fresneda, J., Ribera, I., 2014. Life-history specialization was not an  
653 evolutionary dead-end in Pyrenean cave beetles. *Proc. R. Soc. London B Biol. Sci.*  
654 281, 20132978.

655 Cioffi, R., Moody, A.J., Millán, A., Billington, R.A., Bilton, D.T., 2016. Physiological  
 656 niche and geographical range in European diving beetles (Coleoptera: Dytiscidae).  
 657 Biol. Lett. 12, 20160130.

658 Cooper, S.J.B., Ibrahim, K.M., Hewitt, G.M., 1995. Postglacial expansion and genome  
 659 subdivision in the European grasshopper *Chorthippus parallelus*. Mol. Ecol. 4, 49–  
 660 60.

661 Dawson, A.G., 2013. Ice age Earth: late quaternary geology and climate. Routledge,  
 662 New York.

663 Deffontaine, V., Libois, R., Kotlík, P., Sommer, R., Nieberding, C., Paradis, E., Searle,  
 664 J.B., Michaux, J.R., 2005. Beyond the Mediterranean peninsulas: evidence of  
 665 central European glacial refugia for a temperate forest mammal species, the bank  
 666 vole (*Clethrionomys glareolus*). Mol. Ecol. 14, 1727–1739.

667 Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian coalescent  
 668 inference of past population dynamics from molecular sequences. Mol. Biol. Evol.  
 669 22, 1185–1192.

670 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics  
 671 with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

672 Dynesius, M., Jansson, R., 2000. Evolutionary consequences of changes in species'  
 673 geographical distributions driven by Milankovitch climate oscillations. Proc. Natl.  
 674 Acad. Sci. USA. 97, 9115–9120.

675 Ergin, M., Uluadam, E., Sarikavak, K., Keskin, Ş., Gökaşan, E., Tur, H., 2007. Late  
 676 Quaternary sedimentation and tectonics in the submarine Şarköy Canyon, western  
 677 Marmara Sea (Turkey). Geol. Soc. London, Spec. Publ. 291, 231–257.

678 Fery, H., 2015. *Oreodytes angelinii*, a new species from north-eastern Greece  
 679 (Coleoptera: Dytiscidae: Hydroporinae). Klapalekiana 51, 39–47.

680 Foster, G.N., 1992. Some aquatic Coleoptera from inner Hordaland, Norway. Fauna  
 681 Norv. Ser. B. 39, 63–67.

682 Foster, G.N., Bilton, D.T., Nelson, B.H. 2016. Atlas of the predaceous water beetles  
 683 (Hydradephaga) of Britain and Ireland. FSC Publications, Telford, UK.

684 Foster, G.N., Friday, L.E., 2011. Key to adults of the water beetles of Britain and  
 685 Ireland (Part 1). Handbook for the Identification of British Insects. Vol. 4, Part 5.  
 686 F. Stud. Counc. R. Entomol. Soc. London, London.

687 Franciscolo, M.E., 1979. Fauna d'Italia, Vol. XIV: Coleoptera. Haliplidae, Hygrobiidae,  
 688 Gyrinidae, Dytiscidae. Edizioni Calderini, Bologna.

689    García - Vázquez, D., Bilton, D.T., Alonso, R., Benetti, C.J., Garrido, J., Valladares,  
690        L.F., Ribera, I., 2016. Reconstructing ancient Mediterranean crossroads in  
691        *Deronectes* diving beetles. J. Biogeogr. 43, 1533–1545.

692    García-Vázquez, D., Ribera, I., 2016. The origin of widespread species in a poor  
693        dispersing lineage (diving beetle genus *Deronectes*). PeerJ 4, e2514.

694    Grichuk, V.P., 1984. Late Pleistocene vegetation history. In: Velichko, A.A., Wright Jr,  
695        H.E., Barnosky, C.W. (Eds.), Late Quaternary environments of the Soviet Union.  
696        Longman, London, pp. 155–178..

697    Gündüz, İ., Jaarola, M., Tez, C., Yenyurt, C., Polly, P.D., Searle, J.B., 2007.  
698        Multigenic and morphometric differentiation of ground squirrels (*Spermophilus*,  
699        *Scuiridae*, Rodentia) in Turkey, with a description of a new species. Mol.  
700        Phylogenet. Evol. 43, 916–935.

701    Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003. Barcoding animal life:  
702        cytochrome c oxidase subunit 1 divergences among closely related species. Proc.  
703        R. Soc. London B Biol. Sci. 270, S96–S99.

704    Hewitt, G.M., 1996. Some genetic consequences of ice ages, and their role in  
705        divergence and speciation. Biol. J. Linn. Soc. 58, 247–276.

706    Hewitt, G.M., 1999. Post- glacial re- colonization of European biota. Biol. J. Linn. Soc.  
707        68, 87–112.

708    Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907–913.

709    Hidalgo-Galiana, A., Ribera, I., 2011. Late Miocene diversification of the genus  
710        *Hydrochus* (Coleoptera, Hydrochidae) in the west Mediterranean area. J Biogeogr  
711        59, 377–385.

712    Hidalgo-Galiana, A., Sánchez-Fernández, D., Bilton, D.T., Cieslak, A., Ribera, I., 2014.  
713        Thermal niche evolution and geographical range expansion in a species complex of  
714        western Mediterranean diving beetles. BMC Evol. Biol. 14, 187.

715    Huntley, B., 1988. Glacial and Holocene vegetation history: Europe. Veg. Hist. 341–  
716        383.

717    Jäch, M.A., 1995. The *Hydraena* (*Haenydra*) *gracilis* Germar species complex (Insecta:  
718        Coleoptera: Hydraenidae). Ann. Naturhist. Mus. Wien. 97B, 177–190.

719    Jäch, M.A., 1997. Daytime swarming of rheophilic water beetles in Austria (Coleoptera:  
720        Elmidae, Hydraenidae, Haliplidae). Latissimus 9, 10–11.

- 721 Jäch, M.A., 2015. Hydraenidae. In: Löbl, I., Löbl, D. (Eds.), Catalogue of Palaearctic  
722 Coleoptera, 2nd ed. Volume 2/1: Hydrophiloidea - Staphylinoidea.. Brill, Leiden,  
723 pp. 130–162.
- 724 Jäch, M.A., Balke, M., 2008. Global diversity of water beetles (Coleoptera) in  
725 freshwater. *Hydrobiologia* 595, 419–442.
- 726 Jackson, D.J., 1952. Observations on the capacity for flight of water beetles. *Proc. R.*  
727 *Ent. Soc. Lond.* 27, 57–70.
- 728 Jackson, D.J., 1956. The capacity for flight of certain water beetles and its bearing on  
729 their origin in the western Scottish isles. *Proc. Linn. Soc. Lond.* 167, 76–96.
- 730 Karaman, B., Kıyak, S., Darılmaz, M.C., 2008. Faunistic study of the aquatic beetles  
731 (Coleoptera) of Trabzon province (Turkey). *Mun. Ent. Zool* 3, 437–446.
- 732 Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence  
733 alignment program. *Brief. Bioinform.* 9, 286–298.
- 734 Korkmaz, E.M., Lunt, D.H., Çıplak, B., Değerli, N., Başbüyük, H.H., 2014. The  
735 contribution of Anatolia to European phylogeography: the centre of origin of the  
736 meadow grasshopper, *Chorthippus parallelus*. *J. Biogeogr.* 41, 1793–1805.
- 737 Kotlík, P., Deffontaine, V., Mascheretti, S., Zima, J., Michaux, J.R., Searle, J.B., 2006.  
738 A northern glacial refugium for bank voles (*Clethrionomys glareolus*). *Proc. Natl.*  
739 *Acad. Sci. USA* 103, 14860–14864.
- 740 Kullman, L., 1998. Non- analogous tree flora in the Scandes Mountains, Sweden,  
741 during the early Holocene- macrofossil evidence of rapid geographic spread and  
742 response to palaeoclimate. *Boreas* 27, 153–161.
- 743 Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined  
744 selection of partitioning schemes and substitution models for phylogenetic  
745 analyses. *Mol. Biol. Evol.* 29, 1695–1701.
- 746 Larson, D.J., 1990. *Oreodytes obesus* (Leconte) and *O. sanmarkii* (CR  
747 Sahlberg)(Coleoptera: Dytiscidae) in North America. *Coleopt. Bull.* 44, 295–303.
- 748 Larson, D.J., Alarie, Y., Roughley, R.E., 2000. Predaceous diving beetles (Coleoptera:  
749 Dytiscidae) of the Nearctic Region, with emphasis on the fauna of Canada and  
750 Alaska. NRC Research Press, Ottawa.
- 751 Lozek, V., 2006. Last Glacial paleoenvironments of the West Carpathians in the light of  
752 fossil malacofauna. *J. Geol. Sci. Anthropozoic* 26, 73–84.
- 753 Massilani, D., Guimaraes, S., Brugal, J.P., Bennett, E.A., Tokarska, M., Arbogast,  
754 R.M., Baryshnikov, G., Boeskorov, G., Castel, J.C., Davydov, S. 2016. Past



755 climate changes, population dynamics and the origin of Bison in Europe. BMC  
 756 Biol. 14, 93.

757 Mayr, E., 1970. Populations, species, and evolution: an abridgment of animal species  
 758 and evolution. Harvard University Press, Harvard (MA).

759 Millán, A., Sánchez-Fernández, D., Abellán, P., Picazo, F., Carbonell, J.A., Lobo, J.M.,  
 760 Ribera, I., 2014. Atlas de los coleópteros acuáticos en la España peninsular.  
 761 Ministerio de Agricultura, Alimentación y Medio Ambiente, Centro de  
 762 Publicaciones, Madrid.

763 Nicholls, J.A., Challis, R.J., Mutun, S., Stone, G.N., 2012. Mitochondrial barcodes are  
 764 diagnostic of shared refugia but not species in hybridizing oak gallwasps. Mol.  
 765 Ecol. 21, 4051–4062.

766 Nilsson, A.N., 2001. World catalogue of insects. Volume 3: Dytiscidae (Coleoptera).  
 767 Apollo Books, Stenstrup, DK.

768 Nilsson, A.N., Hájek, J. 2017a. A World Catalogue of the Family Dytiscidae , or the  
 769 Diving Beetles ( Coleoptera , Adephaga ). Update distributed as a PDF file via  
 770 Internet; version 31.I.2017; Available at: <http://www.waterbeetles.eu>

771 Nilsson, A.N., Hájek, J., 2017b. Catalogue of Palearctic Dytiscidae (Coleoptera).  
 772 Update distributed as a PDF file via Internet; version 1. I. 2017. Available at:  
 773 <http://www.waterbeetles.eu>

774 Nilsson, A.N., Holmen, M., 1995. Fauna Entomol. Scandinaviaca V. 32. The Aquatic  
 775 Adephaga (Coleoptera) of Fennoscandia and Denmark. II. Dytiscidae. Brill,  
 776 Leiden.

777 Nilsson, A.N., Kholin, S., 1994. The diving beetles (Coleoptera, Dytiscidae) of  
 778 Sakhalin-an annotated checklist. Entomol. Tidskr. 115, 143–156.

779 Ponel, P., Guiter, F., Gandouin, E., Pailles, C., Rioual, P., Djamali, M., Andrieu-Ponel,  
 780 V., Leydet, M., Van der Putten, N., de Beaulieu, J.L., 2016. Novel insights from  
 781 coleopteran and pollen evidence into the Lateglacial/Holocene transition in  
 782 Aubrac, French Massif Central. Palaeogeogr. Palaeoclimatol. Palaeoecol. 463, 83–  
 783 102.

784 Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1. 6. 2014.  
 785 Available at <http://beast.bio.ed.ac.uk/Tracer>.

786 Rand, A.L., 1948. Glaciation, an isolating factor in speciation. Evolution 2, 314–321.

787 Ribera, I., 2003. Are Iberian endemics Iberian? A case-study using water beetles of  
 788 family Dytiscidae (Coleoptera). Graellsia 59, 475–502.

789 Ribera, I., 2008. Chapter 15: Habitat constraints and the generation of diversity in  
790 freshwater macroinvertebrates. In: Lancaster, J., Briers, R.A. (Eds.), Aquatic  
791 Insects: Challenges to Populations. CAB International, UK, pp. 289–311.

792 Ribera, I., Castro, A., Díaz, J.A., Garrido, J., Izquierdo, A., Jach, M.A., Valladares,  
793 L.F., 2011. The geography of speciation in narrow-range endemics of the  
794 “*Haenydra*” lineage (Coleoptera, Hydraenidae, *Hydraena*). J. Biogeogr. 38, 502–  
795 516.

796 Ribera, I., Castro, A., Hernando, C., 2010. *Ochthebius (Enicocerus) aguilerai* sp. n.  
797 from central Spain, with a molecular phylogeny of the Western Palaearctic species  
798 of *Enicocerus* (Coleoptera, Hydraenidae). Zootaxa 2351, 1–13.

799 Ribera, I., Nilsson, A.N., Vogler, A.P., 2004. Phylogeny and historical biogeography of  
800 Agabinae diving beetles (Coleoptera) inferred from mitochondrial DNA  
801 sequences. Mol. Phylogenet. Evol. 30, 545–562.

802 Rocchi, S., 2007. Il genere *Oreodytes* Seidlitz, 1887 negli Appennini. Onychium 5, 29–  
803 33.

804 Rokas, A., Atkinson, R.J., Webster, L., Csóka, G., Stone, G.N., 2003. Out of Anatolia:  
805 longitudinal gradients in genetic diversity support an eastern origin for a circum -  
806 Mediterranean oak gallwasp *Andricus quercustozae*. Mol. Ecol. 12, 2153–2174.

807 Schmitt, T., Varga, Z., 2012. Extra-Mediterranean refugia: The rule and not the  
808 exception? Front. Zool. 9, 22.

809 Sharp, D., 1882. On aquatic carnivorous Coleoptera or Dytiscidae. Sci. Trans. R. Dublin  
810 Soc. 2, 17–1003.

811 Shaverdo, H.V., Fery, H., 2006. *Oreodytes shorti* sp.n. from Mongolia (Coleoptera,  
812 Dytiscidae). Koleopterol. Rundschau 76, 35–42.

813 Silvestro, D., Michalak, I., 2012. RAxMLGUI: a graphical front-end for RAxML. Org.  
814 Divers. Evol. 12, 335–337.

815 Soffer, O., 1990. The Russian Plain at the last glacial maximum. In: Soffer, O., Gamble,  
816 C. (Eds.), The world at 18000 BP: volume 1, high latitudes. Unwin Hyman,  
817 London, pp. 228–252.

818 Sommer, R.S., Nadachowski, A., 2006. Glacial refugia of mammals in Europe:  
819 evidence from fossil records. Mamm. Rev. 36, 251–265.

820 Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the  
821 RAxML web-servers. Syst. Biol. 57, 758–771.

- 822 Stewart, J.R., Lister, A.M., 2001. Cryptic northern refugia and the origins of the modern  
823 biota. *Trends Ecol. Evol.* 16, 608–613.
- 824 Stewart, J.R., Lister, A.M., Barnes, I., Dalén, L., 2009. Refugia revisited: individualistic  
825 responses of species in space and time. *Proc. R. Soc. London B Biol. Sci.* 277,  
826 661–671.
- 827 Tarnowska, E., Niedziałkowska, M., Gerc, J., Korbut, Z., Górny, M., Jędrzejewska, B.,  
828 2016. Spatial distribution of the Carpathian and Eastern mtDNA lineages of the  
829 bank vole in their contact zone relates to environmental conditions. *Biol. J. Linn.*  
830 *Soc.* 119, 732–744.
- 831 Trizzino, M., Audisio, P.A., Antonini, G., Mancini, E., Ribera, I., 2011. Molecular  
832 phylogeny and diversification of the “*Haenydra*” lineage (Hydraenidae, genus  
833 *Hydraena*), a north-Mediterranean endemic-rich group of rheophilic Coleoptera.  
834 *Mol. Phylogenet. Evol.* 61, 772–783.
- 835 Trizzino, M., Carnevali, L., De Felici, S., Audisio, P., 2013. A revision of *Hydraena*  
836 species of the “*Haenydra*” lineage (Coleoptera, Hydraenidae). *Zootaxa* 3607, 1–  
837 173.
- 838 Trizzino, M., Valladares, L.F., Garrido, J., Audisio, P., 2012. Morphological reply to a  
839 DNA call: a new cryptic species of *Hydraena* from western Europe, with a  
840 complete overview of the *Hydraena gracilis* complex (Coleoptera:  
841 Hydraenidae: “*Haenydra*” lineage). *J. Nat. Hist.* 46, 1065–1078.
- 842 Willis, K.J., Rudner, E., Sümegi, P., 2000. The full-glacial forests of central and  
843 southeastern Europe. *Quat. Res.* 53, 203–213.
- 844 Zack, R.S., 1992. Notes on the Genus *Oreodytes* (Coleoptera: Dytiscidae) in the Pacific  
845 Northwest. *Coleopt. Bull.* 46, 151–154.

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## Figure legends

**Figure 1.** Distribution of studied species of the *Hydraena gracilis* complex. White circles, sampled localities.

**Figure 2.** Distribution of studied species of the *Platambus maculatus* complex. White circles, sampled localities.

**Figure 3.** Distribution of *Oreodytes sanmarkii* and *Oreodytes davisii* in the Western Palearctic. Coloured circles, sampled localities for each species, including also *Oreodytes alpinus* and *Oreodytes septentrionalis*. Localities from Mongolia and Siberia for *O. sanmarkii* and *O. alpinus* respectively are not show.

**Figure 4.** Phylogenetic tree of the *Hydraena gracilis* complex. Ultrametric tree obtained with BEAST with combined nuclear and mitochondrial sequences and a partition by gene. Numbers on nodes represent Bayesian posterior probabilities higher than 0.5. See Table S1 for details of specimens and localities. Habitus photograph, *H. gracilis* (Lech Borowiec).

**Figure 5.** Coalescence Skyline plots of A) *H. gracilis*; B) *P. maculatus*; C) *Oreodytes sanmarkii*. Blue lines represent 95% highest probability density; horizontal axis - time before present (Ma); vertical axis - effective population size (NeT).

**Figure 6.** Phylogenetic tree of the *Platambus maculatus* complex. Ultrametric tree obtained with BEAST with combined nuclear and mitochondrial sequences and a partition by gene. Numbers on nodes represent Bayesian posterior probabilities higher than 0.5. See Table S1 for details of specimens and localities. Habitus photographs, from base to tip, north Italian form, *P. maculatus* "graellsii", north-Scottish form, and typical *P. maculatus* (dotted lines mark the corresponding specimens).

**Figure 7.** Phylogenetic tree for studied *Oreodytes* species. Ultrametric tree obtained with BEAST with combined nuclear and mitochondrial sequences and a partition by gene. Numbers on nodes represent Bayesian posterior probabilities higher than 0.5. See

882 Table S1 for details of specimens and localities. Habitus photographs, *O. sanmarkii* (L.  
883 Borowiec) and *O. davisii* (U. Schmidt).

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885 **Figure 8.** Habitus of (A) *Platambus maculatus*, standard form (specimen voucher  
886 MNCN-AH71); (B) form "*graellsii*" (voucher MNCN-AI733); (C) specimen from north  
887 Italy (voucher MNCN-AH191); (D) specimen from the Scottish Lochs (voucher IBE-  
888 AI975). See Table S1 for details on the specimens, and Figs 6, S2 and S3 for the  
889 phylogenetic relationships of the specimens.

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**Table 1.** Clock and coalescent demographic model comparisons for each group, including AICM values and standard errors (SE). Best AICM value for each pair shown in bold; models that failed to converge adequately in brackets or represented by a dash. Differences < 2 units were not considered significant.

group	clock	AICM	SE	coalescence	AICM	SE
<i>H. gracilis</i>	relaxed	[9076.7]	[±0.11]	constant	<b>6705.3</b>	±0.05
	strict	<b>9385.8</b>	±0.24	exponential	6717.2	±0.24
				expansion	[6708.5]	±0.26]
				logistic	-	-
<i>P. maculatus</i>	relaxed	<b>18568.9</b>	±0.52	constant	-	-
	strict	18596.7	±0.44	exponential	[11173.9]	±19.20]
				expansion	[9903.9]	±65.07]
				logistic	-	-
<i>O. sanmarkii</i>	relaxed	<b>23948.4</b>	±0.87	constant	-	-
	strict	23952.8	±0.56	exponential	8204.6	±0.01
				expansion	<b>8200.2</b>	±0.15
				logistic	8202.3	±0.13